

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF :  
Eiichi KITAZONO, ET AL. :EXAMINER: Schmidtmann, Bahar  
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DECLARATION UNDER 37 C.F.R. 1,132

COMMISSIONER FOR PATENTS  
P.O. Box 1450  
ALEXANDRIA, VIRGINIA 22313

Sir:

I, Hiroaki Kaneko, am one of the inventors of the present invention.

I have conducted the following experiments.

Run 1

409 mg (40 equivalents based on 100 equivalents of the carboxyl group of hyaluronic acid) of L- $\alpha$ -dipalmitoylphosphatidyl ethanolamine was dissolved in 200 ml of a 1/1 solution of tetrahydrofuran and water (v/v). 500 mg of sodium hyaluronate was added to this solution, and 0.1M HCl and 0.1M NaOH were added to the resulting solution to adjust its pH to 6.8. 120 mg of a hydrochloric acid salt of 1-ethyl-3-[3-(dimethylamino)propyl]-carbodiimide (WSC) and 100 mg of 1-hydroxybenzotriazole (HOBT) were dissolved in 10 ml of a 1/1 solution of tetrahydrofuran and water, and the resulting solution was added to a reaction system and stirred through the night. After stirring, the obtained solution was purified by dialysis and freeze dried to obtain a product of interest.

30 mg of this freeze dried product was dissolved in 970 mg of ion exchange water to form a non-viscous liquid having a concentration of 3 wt%.

Run 2

The procedures of Run 1 was repeated except that 443 mg (40 equivalents based on 100 equivalents of the carboxyl group of hyaluronic acid) of L- $\alpha$ -distearoylphosphatidyl ethanol amine was used instead of 409mg of L- $\alpha$ -dipalmitoylphosphatidyl ethanolamine.

10 mg of the freeze dried product was dissolved in 990

mg of ion exchange water to form a non-viscose liquid having a concentration of 1 wt%.

Run 3

30 mg of the freeze dried product which was obtained in the above Run 2 was dissolved in 970 mg of ion exchange water to form a hydrogel having a concentration of 3 wt%.

A complex elastic modulus of the hydrogel was 47 Pa.

The complex elastic modulus (i.e. 47Pa) is clearly lower than that (i.e. 902Pa) of the hydrogel of the hyaluronic acid conjugated with phosphatidyl ethanolamine bearing an unsaturated oleyl group obtained in Example 2 of the present application.

It is understood from the above experiments that the hyaluronic acid conjugated with phosphatidyl ethanolamine bearing a saturated stearoyl group i.e. C<sub>18</sub> saturated fatty acid (Runs 2 and 3) as well as the hyaluronic acid conjugated with phosphatidyl ethanolamine bearing a saturated palmitol group i.e. C<sub>16</sub> saturated fatty acid (Run 1) do not show an unique complex elastic modulus of the hyaluronic acid conjugated with phosphatidyl ethanolamine bearing an unsaturated oleyl group (i.e. C<sub>18</sub> unsaturated fatty acid) of the present invention.

The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Further declarant saith not.

Shoaki Kase/co  
Signature

Aug. 22, 2011

Date